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This DER was originally prepared under contract by Dynamac Corporation (2275 Research Boulevard, Suite 300; Rockville, MD 20850; submitted 10/28/2008). The DER has been reviewed by the Health Effects Division (HED) and revised to reflect current Office of Pesticide Programs (OPP) policies.

## STUDY REPORT:

47453707 Banijamali, A. (2007) Distribution and Metabolism of (Carbon-14)-Bifenazate in Corn. Project Number: 2006/026, R170601. Unpublished study prepared by Chemtura Corporation and Research for Hire. 230 p.

## EXECUTIVE SUMMARY:

Chemtura has submitted a study investigating the metabolism of [U-anisole ring-$^{14}$C]bifenazate (specific activity 3.38 mCi/mmol) in corn. The radiolabeled test substance was formulated using the inert ingredients of Acramite®-4SC and diluted with water for over-the-top foliar spray application to field corn grown outdoors at a nominal rate of 0.75 lb ai/A or an exaggerated rate of 5 lb ai/A. The spray treatment was made to corn plants 33 days after seed planting and when the plants were about 18 inches in height. Immature forage was harvested 5 days after treatment (DAT), and mature stover and grain were harvested 104 DAT. The collected samples were stored frozen and extracted within 21-29 days (~3-4 weeks) for forage and 83 days (~12 weeks) for stover. Information pertaining to sample analysis dates was not provided but the petitioner reported that initial chromatographic analyses were completed within 5 months of harvest.

Total radioactive residues (TRR; expressed as bifenazate equivalents) in/on field corn matrices were determined by combustion/LSC. TRR for samples treated at 0.75 lb were 8.52 ppm in forage, 0.492 in stover, and 0.0117 ppm in grain. At the exaggerated rate of 5.0 lb ai/A, TRR were 65.3 ppm in forage, 3.16 in stover, and 0.0947 ppm in grain. These TRR data show that [$^{14}$C]bifenazate sprayed on corn plants was poorly translocated to grain.

Adequate procedures were employed to fractionate and characterize/identify radioactive residues. Extraction with various organic solvents released the majority of the radioactivity (~79-84% TRR) from corn forage and stover. Soxhlet extraction with methanol and methanol/water released only 24.7-45.3% TRR from grain; the percentages of residues initially

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extracted from grain may seem marginal, but it is noted that TRR in grain were low to begin with. Further hydrolysis with enzymes, acid and/or base released an additional 1.9-8.8% TRR from forage, 13.7-14.2% TRR from stover, and 61.3% TRR from grain which received the exaggerated rate treatment; base hydrolysis generally released the largest amount of radioactivity. Nonextractable residues were <6% TRR in all samples following hydrolysis. Overall accountabilities of residues from all corn matrices ranged ~90-95%. Residues were identified and quantitated by HPLC, and metabolite identities were confirmed in forage by HPLC comparison with reference standards and LC/MS. Furthermore, LC/MS SIM analysis was conducted to detect structurally related hydroxy biphenyl residues.

The following major residue components were identified in/on various matrices following treatment at the nominal rate (the corresponding value for the exaggerated rate treatment is in parenthesis). The parent, bifenazate, was a principal residue in forage at 15.1% TRR (5.4% TRR) and stover at 2.4% TRR (6.1% TRR). The diazene metabolite (designated as D23-06 in the current study but has been determined to be the same regulated metabolite D3598 from previous metabolism studies), was detected in forage at 8.5% TRR (11.8% TRR) and stover at 6.5% TRR (10.0% TRR). The carbamate metabolite (D23-04) was present in forage at 9.7% TRR (13.0% TRR) and stover at 7.6% TRR (14.3% TRR). Metabolite D23-19 (or A1530S) was identified in forage at 4.7% TRR (3.7%) and stover at 11.8% TRR (8.2% TRR). Metabolite D23-19 was the only residue identified in exaggerated rate grain at 1.4% TRR.

Other minor metabolites identified in forage and stover included D23-08 (2-4% TRR), D23-13 (3-4% TRR); D23-16 (or A1530; 2-4% TRR); D23-03 (1-4% TRR); D-23-02 (or D1989; 6-8% TRR); and D23-07 (3% TRR). In addition, (4-methoxybiphenyl-3-yl)carbamate was detected in forage (~10-13% TRR) and stover (8-14% TRR) but was determined to be an impurity in the dosing solution. A single unknown detected in both forage and stover, accounting for up to 11% TRR in forage, was a broad peak eluting at the end of the chromatographic run; no single region of radioactivity was detected and the petitioner reported that this peak represents the column wash (100% ACN). Polar unknowns eluting near the solvent front accounted for 19.9% TRR in grain and up to 8% TRR in forage and stover. Attempts were made to retain the polar peak using a Hypercarb HPLC column; however, based on the mass spectral data and lack of a corresponding peak, no structure could be proposed but the peak was characterized as a highly polar metabolite. LC/MS SIM analysis of the high-rate forage extract confirmed that biphenyl hydrazine was not present in corn forage. HPLC analysis of the enzyme hydrolysate (~1-2% TRR) and base hydrolysate (6.5% TRR) of forage showed the majority of the radioactivity being extremely polar, and eluting at or near the solvent front.

Based on the results of the study, the petitioner concluded that the metabolism of bifenazate in corn proceeds via oxidation of the hydrazine moiety of bifenazate to form the diazene (D23-06 or D3598) which is further oxidized to the diazene oxide (D23-07) and then degraded by oxidative/hydrolysis of the side chain yielding 4-methoxybiphenyl (D23-02), 3-aminobiphenyl-4-ol (D23-08), biphenyl-3,4-diol (D23-13), biphenyl-4-ol (D23-16), 4-methoxybiphenyl-3-amine (D23-03), and biphenyl-4-yl-sulfate (D23-10).

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## STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the plant metabolism data are classified as scientifically acceptable. The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP# 355504.

[Note to EPA: We considered requesting additional storage stability data for this study but decided against it. The companion storage stability data (refer to the DER for MRIDs 47453713, 47467703, and 47467705) show that in forage, residues declined by ~74 % after 4 weeks of frozen storage. Since the metabolites identified in this study are consistent with those found in previous studies (apple, orange, and cotton), the regulatory conclusions regarding the residues of concern will probably not change even if we ask for storage stability data. We will definitely correct the residues reported in corn field trials for decline in residues. Dynamac]

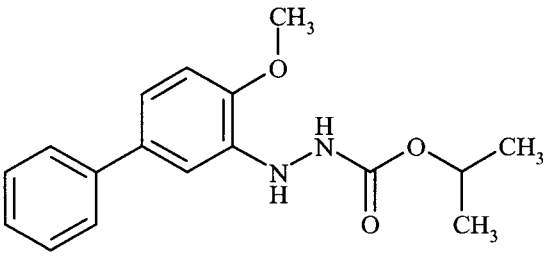
## COMPLIANCE:

Signed and dated Good Laboratory Practice (GLP), Quality Assurance and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported which would have an impact on the validity of the study.

## A. BACKGROUND INFORMATION

Bifenazate is a selective miticide which controls the motile stage of mites either by direct contact or through contact with foliar residues. Bifenazate blocks or closes the gamma-aminobutyric acid (GABA) activated chloride channels of susceptible pests resulting in over-excitation of the peripheral nervous system. The study submission reviewed in this DER was submitted in support of a request to register Acramite®-4SC (EPA Reg. No. 400-514) for use on field and sweet corn. The chemical structure and nomenclature of bifenazate and the physicochemical properties of the technical grade of bifenazate are presented in Tables A.1 and A.2.

**Table A.1. Test Compound Nomenclature.**

| | |
|---|---|
| Compound |  |
| Common name | Bifenazate |
| Company experimental name | D2341 |
| IUPAC name | isopropyl 2-(4-methoxybiphenyl-3-yl)hydrazinoformate |
| CAS name | 1-methylethyl 2-(4-methoxy[1,1'-biphenyl]-3-yl)hydrazinecarboxylate |
| CAS registry number | 149877-41-8 |
| End-use products (EPs) | Acramite®-4SC (4 lb/gal FlC; EPA Reg. No. 400-514) |

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| Table A.2. Physicochemical Properties of the Technical Grade of Bifenazate. | | |
|---|---|---|
| Melting range | 124-125 °C | MRID 46064101 |
| pH | 6.78 | |
| Density | 1.19 g/cm$^3$ | |
| Water solubility | 2.1 mg/L (20 °C) | |
| Solvent solubility | 102 mg/mL ethyl acetate (20 °C) | |
| Vapor pressure | <1 x $10^{-8}$ atm M$^3$/mole (25 °C) | |
| Dissociation constant, $pK_a$ | 12.94 at 23 °C | |
| Octanol/water partition coefficient, Log($K_{OW}$) | 3.4 | |
| UV/visible absorption spectrum | Max 264 nm in water | |

# B. EXPERIMENTAL DESIGN

## B.1. Test Site and Crop Information

The field test sites were located at Research For Hire (Porterville, CA). A summary of the test site information is provided in Table B.1.1, and a summary of the crop information is provided in Table B.1.2.

Field corn was planted and grown outdoors in raised boxes. Three plots were established, one plot for each treatment rate, and one plot for untreated (control) plants; each plot area was 15 ft$^2$. The plots were maintained following normal agricultural practices, and maintenance fertilizers and pesticides were applied during the study period. For the preceding 3 years, the soil was fallow with no chemical applications made. Monthly weather conditions (rainfall and temperature) were reported. The petitioner stated that air temperatures and precipitation were normal during the study period.

**TABLE B.1.1. Test Site Information.**

| Type | Method | Soil characteristics | | | |
|---|---|---|---|---|---|
| | | Type | %OM | pH | CEC (meq/100 g) |
| Foliar treatment to plants grown in raised boxes outdoors | Over-the-top spray application | sandy clay loam | 2.2 | 7.5 | 28.1 |

**TABLE B.1.2. Crop Information.**

| Crop; crop group | Variety | Growth stage at application | Growth stage at harvest | Harvested Matrix |
|---|---|---|---|---|
| Field Corn; Grain, cereal, group 15, and Grain, cereal, forage, fodder, and straw, group 16 | Hybrid N8214 | 33 days after planting of seed; 18 inches tall | Immature: 5 DAT<br>Mature: 104 DAT | Forage<br>Stover and grain |

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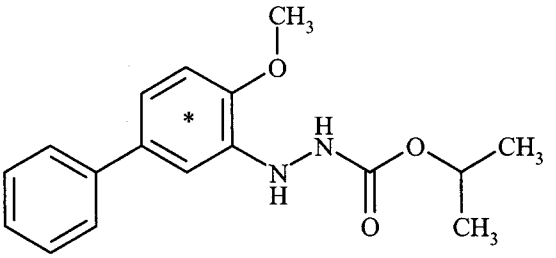
## B.2. Test Materials

The radiolabeled test substance ($^{14}$C-bifenazate) was mixed with $^{12}$C-bifenazate to achieve the desired specific activity and formulated as a suspension concentrate, with Acramite®-4SC inerts. The characteristics of the test substance are presented in Table B.2.1.

**TABLE B.2.1. Test Material Characteristics.**

| | |
|---|---|
| Chemical structure |  |
| Radiolabel position | [U-anisole ring-$^{14}$C]bifenazate |
| Lot No. | EPPS-06-045-85-11 |
| Purity | 99.4 |
| Specific activity | 3.38 mCi/mmol (24,980 dpm/µg dosing formulation) |

## B.3. Study Use Pattern

The formulated test substance was diluted with water for foliar application at a target rate of 0.75 lb ai/A or 5 lb ai/A (exaggerated rate) to field corn, 33 days after planting of seed. Spray applications were made using a hand sprayer. Details of the study use pattern are presented in Table B.3.1.

**TABLE B.3.1. Use Pattern Information.**

| | |
|---|---|
| Chemical name | [U-anisole ring-$^{14}$C]bifenazate |
| Application method | The test substance formulated as a FlC was diluted with water for application using a spray trigger and nozzle. |
| Application rate | Nominal rate: 0.75 lb ai/A; Exaggerated rate: 5.0 lb ai/A |
| Number of applications | 1 |
| Timing of applications | 33 days after planting of seed |
| PHI | immature forage: 5 days; mature stover and grain: 104 days |

## B.4. Identification/Characterization of Residues

### B.4.1. Sample Handling and Preparation

Following harvest, forage, stover and grain samples were ground, placed in residue bags and stored frozen. Frozen samples were shipped on dry ice to Chemtura Corporation (Middlebury, CT) for analysis. Prior to extraction, samples were re-homogenized to obtain a uniformly homogenized mixture. Nominal- and exaggerated-rate treated samples of forage, stover and grain were extracted.

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Briefly, homogenized forage and stover samples were extracted 3-5 times with acetonitrile (ACN) and centrifuged. Remaining solids in forage were then sequentially extracted with ACN:aqueous ammonium acetate (1:1, v:v), ACN:methanol:water (1:1:1, v:v:v), 100% methanol, and lastly water. Remaining solids in stover were sequentially extracted with ACN:water (6x; 75:25 and 50:50, v:v), water (3x), and ethyl acetate (1x). Each extract was collected with centrifugation. The extract groups were combined and concentrated prior to TLC and/or SPE cleanup and HPLC analysis.

Homogenized grain samples were soxhlet extracted with methanol, then methanol:water (1:1,v:v) for 24 hours.

Nonextractable residues of forage and stover (both treatment rates), and grain (exaggerated rate only) were subjected to enzyme and acid and/or base hydrolysis. Nonextractable residues in 0.1 M sodium citrate buffer (pH 5) were mixed with β-glucosidase, cellulose, and pectinex enzymes and incubated at 37-40 °C for 72 hours. The hydrolysate was concentrated and treated with ACN to precipitate proteins prior to HPLC analysis. Following enzyme-hydrolysis, nonextractable residues were sequentially hydrolyzed with 1 N HCl and 1 N NaOH (except nominal-rate forage) at 40 °C overnight.

### B.4.2. Analytical Methodology

TRR were determined in the samples by combustion/LSC. TRR in the extracts and hydrolysates was determined by direct LSC, and radioactivity in the nonextractable solids was determined by combustion/LSC. The limit of detection for combustion/LSC was reported at 0.5 ppb.

The solvent extracts of corn forage, stover and grain, and enzyme and base hydrolysates of forage only were analyzed by HPLC. The HPLC system utilized a phenyl-hexyl column, UV detection, in-line radiodetection, and a gradient mobile phase of 50 mM ammonium acetate in water (pH 4) and ACN. Fractions were collected and metabolites were quantitated by LSC; HPLC radiochromatograms were reconstructed.

Residues in the extracts of forage and/or stover were isolated using solid phase extraction (SPE) or TLC. The forage extract was concentrated and redissolved in hexane and applied to a Florisil SPE column; residues were sequentially eluted with several volumes of hexane, ethyl acetate:hexane (5:95, 10:90, 20:80, 50:50, v:v), ethyl acetate and methanol. The fractions were concentrated for HPLC analysis. A separate aliquot of the concentrated forage (exaggerated rate) extract was applied to a preparative TLC silica gel plate and developed in ACN:water (75:25, v:v). Radioactivity was visualized with phosphor-imaging and radioactive bands were collected with ACN, filtered and concentrated for HPLC analysis. Further separation of the collected SPE or TLC fractions was conducted using an HPLC system similar to the one described above, except that a mobile phase of water and ACN was used. Isolated residues were then concentrated for metabolite identification by LC/MS.

Mass spectral identification of the metabolites used a multi-step approach. First, isolated fractions were chromatographed on an Agilent Eclipse column using a mobile phase of water and

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acetonitrile, each with 0.3% formic acid. The effluent was passed through a radiodetector and then mass spectrometer. From the full-scan positive or negative ion electrospray ionization (ESI) or positive atmospheric pressure chemical ionization/atmospheric pressure photo ionization (APCI/APPI) spectra the $[M+H]^+$ or $[M-H]^-$ psuedo-molecular ion of a radioactive metabolite was determined. Secondly, these ions were fragmented by collision induced dissociation (CID); the full scan mass spectra displayed ions from the dissociation of only selected precursor ions. Single ion monitoring mass spectrophotometry was used for detection/identification of low abundant metabolites and to determine biphenyl hydrazine and related amino biphenyl compounds directly in the forage and stover extracts.

Metabolite identifications in forage were confirmed by comparison of HPLC retention times and mass spectra with those of unlabeled reference standards. Identifications in stover were made by HPLC co-chromatography with the isolated and identified forage metabolite; the parent in stover was confirmed by co-chromatography of the extract with radiolabeled standard. Standards used in the study are presented in Appendix I.

## C. RESULTS AND DISCUSSION

The storage conditions and durations for corn matrices are presented in Table C.1. The petitioner provided sampling and extraction dates for forage and stover samples, and stated that initial quantitation of bifenazate and related metabolites took place within 5 months of harvest.

Following over-the-top foliar application of [U-anisole ring-$^{14}$C]bifenazate at 0.75 lb ai/A, TRR in field corn matrices (Table C.3.1.), determined by combustion/LSC, were 8.52 ppm in forage harvested 5 DAT, and 0.492 and 0.0117 ppm in mature stover and grain, respectively, harvested 104 DAT. At the exaggerated application rate of 5.0 lb ai/A, TRR were 65.3 ppm in forage harvested 5 DAT, and 3.16 and 0.0947 ppm in mature stover and grain, respectively, harvested 104 DAT.

The distribution of radioactivity in corn matrices is presented in Tables C.2.2.1 (nominal application rate of 0.75 lb ai/A) and C.2.2.2 (exaggerated application rate of 5.0 lb ai/A). Extraction with various organic solvents released the majority of the radioactivity (~79-84% TRR) from corn forage and stover. Soxhlet extraction with methanol and methanol/water released only 24.7-45.3% TRR from grain; the percentages of residues initially extracted from grain may seem marginal, but it is noted that TRR in grain were low to begin with. Further hydrolysis with enzymes, acid and/or base released an additional 1.9-8.8% TRR from forage, 13.7-14.2% TRR from stover, and 61.3% TRR from grain which received the exaggerated rate treatment; base hydrolysis generally released the largest amount of radioactivity. Nonextractable residues were <6% TRR in all samples following hydrolysis. Overall accountabilities of residues from all corn matrices ranged ~90-95%. Residues were identified and quantitated by HPLC, and metabolite identities were confirmed in forage by HPLC comparison with reference standards and LC/MS. Furthermore, LC/MS SIM was conducted to detect structurally related hydroxy biphenyl residues.

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The characterization and identification of residues in corn matrices are summarized in Tables C.2.3.1 (nominal application rate) and C.2.3.2 (exaggerated application rate). Although the same metabolites were identified in the nominal- and exaggerated- rate samples, the percent TRR varied between the respective samples. The parent, bifenazate, was the major residue identified in forage (nominal rate) at 15.1% TRR (1.29 ppm), but was a minor residue in exaggerated-rate forage at 5.4% TRR (3.54 ppm), and in stover at 2.4% TRR (0.012 ppm) and 6.1% TRR (0.189 ppm) treated at the nominal and exaggerated rates, respectively. The diazene metabolite (previously identified as D3598 and determined a residue of concern in plants) was identified as a minor residue in forage at 8.5% TRR (0.726 ppm) and stover at 6.5% TRR (0.032 ppm) treated at the nominal rate. However, diazene represented a larger amount of the radioactivity (11.8% and 10.0% TRR) in forage and stover treated at the exaggerated rate. The major residue identified in forage and stover at the exaggerated rate was the carbamate metabolite accounting for 13.0% and 14.3% TRR, respectively; levels were lower (9.7% and 7.6% TRR, respectively) in forage and stover treated at the nominal application rate. The only other major metabolite identified was biphenyl-4-yl-sulfate at 11.8% TRR in stover (nominal rate); bipheyl-4-yl-sulfate was a minor residue in exaggerated-rate stover accounting for 8.2% TRR and in forage (both rates) at 3.7-4.7% TRR. Biphenyl-4-yl-sulfate was the only residue identified in grain (exaggerated rate) at very low levels (1.4% TRR, 0.001 ppm).

Other minor metabolites identified in forage and stover included 3-aminobiphenyl-4-ol (2-4% TRR); biphenyl-3,4-diol (3-4% TRR); biphenyl-4-ol (2-4% TRR); 4-methoxybiphenyl-3-amine (1-4% TRR); 4-methoxybiphenyl (6-8% TRR); and diazene oxide (3% TRR). In addition, (4-methoxybiphenyl-3-yl)carbamate was detected in forage (~10-13% TRR) and stover (8-14% TRR) but was determined to be an impurity in the dosing solution. Positive ion ESI LC/MS/MS of the dosing solution indicated the presence of (4-methoxybiphenyl-3-yl)carbamate.

A single unknown detected in both forage and stover, accounting for up to 11% TRR in forage, was a broad peak eluting at the end of the chromatographic run; no single region of radioactivity was detected and the petitioner states this peak represents the column wash (100% ACN). Polar unknowns eluting near the solvent front accounted for 19.9% TRR (0.019 ppm) in grain and up to 8% TRR in forage and stover. Attempts were made to retain the polar peak using a Hypercarb HPLC column; however, based on the mass spectral data and lack of a corresponding peak, no structure could be proposed but the peak was characterized as a highly polar metabolite. LC/MS SIM of the high-rate forage extract confirmed that biphenyl hydrazine was not present in treated corn forage.

HPLC analysis of the enzyme hydrolysate (~1-2% TRR) and base hydrolysate (6.5% TRR) of forage showed the majority of the radioactivity being extremely polar, and eluting at or near the solvent front. The enzyme-released radioactivity was determined identical to the polar components in the forage extracts, and the base-released residues of exaggerated-rate forage consisted of multiple polar components (each ≤1.6% TRR) and the remainder of the radiochromatogram indicated no single region of radioactivity. Due to low radioactivity and the inability to concentrate the enzyme and base hydrolysates of stover and grain, HPLC analyses were not conducted on these samples.

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## C.1. Storage Stability

The petitioner reported that harvested samples, homogenized samples, and extracts were stored in freezers and refrigerators throughout the study, and that forage and stover extractions were completed within 3-12 weeks of harvest, with HPLC analyses completed 2-8 weeks later. Further experimental work for metabolite identification was completed within 1 year of harvest.

Based on the study dates provided, including extraction dates reported on the raw data pages, it appears that samples were extracted within 21-29 days (~3-4 weeks) for forage and 83 days (~12 weeks) for stover. Actual analysis dates were not provided for forage or stover, and extraction dates were not provided for grain.

**TABLE C.1. Summary of Storage Conditions.**

| Matrix | Storage Temperature (°C) | Actual Storage Duration [1] | Interval of Demonstrated Storage Stability |
|---|---|---|---|
| Corn, forage | Samples frozen; extracts frozen or refrigerated | 21-29 days to extraction | None provided. |
| Corn, stover | | 83 days to extraction | |
| Corn, grain | | No extraction date | |

[1] Duration from harvest to extraction; analysis dates were not provided.

## C.2. Identification, Characterization, and Distribution of Residues

**TABLE C.2.1. Total Radioactive Residues (TRR) in Field Corn Matrices.**

| Matrix | Timing and Applic. No. | PHI (days) | Application Rate | |
|---|---|---|---|---|
| | | | 0.75 lb ai/A | 5.0 lb ai/A |
| | | | ppm | ppm |
| Forage | A single foliar spray application, 33 days after planting of seed | 5 | 8.52 | 65.3 |
| Stover | | 104 | 0.492 | 3.16 |
| Grain | | 104 | 0.0117 | 0.0947 |

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**TABLE C.2.2.1. Distribution of the Parent and the Metabolites in Corn Matrices Following Application of [U-anisole ring-$^{14}$C]Bifenazate at 0.75 lb ai/A.[1]**

| Metabolite Fraction | Corn, Forage | | Corn, Stover | | Corn, grain | |
|---|---|---|---|---|---|---|
| | TRR = 8.52 ppm | | TRR = 0.492 ppm | | TRR = 0.0117 ppm | |
| | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| ACN extracts | 79.39 | NR | 42.25 | NR | | |
| ACN/ammonium acetate extract | 2.87 | NR | | | | |
| ACN/MeOH/water extract | 1.35 | NR | | | | |
| MeOH extract | 0.82 | NR | | | | |
| ACN/water extracts | | | 28.88 | NR | | |
| Water extracts | | | 7.21 | NR | | |
| Ethyl acetate extract | | | 0.57 | NR | | |
| -Total extracts | 84.4 | 7.19 | 78.9 | 0.388 | | |
| Bifenazate | 15.1 | 1.29 | 2.44 | 0.012 | | |
| Diazene (D3598) [2] | 8.52 | 0.726 | 6.49 | 0.032 | | |
| Biphenyl-4-yl-sulfate | 4.69 | 0.400 | 11.8 | 0.058 | | |
| 3-Aminobiphenyl-4-ol | 1.98 | 0.169 | 3.38 | 0.017 | | |
| Biphenyl-3,4-diol | 3.43 | 0.292 | 4.22 | 0.021 | | |
| Biphenyl-4-ol | 3.54 | 0.301 | 2.36 | 0.012 | | |
| 4-Methoxybiphenyl-3-amine | 2.46 | 0.209 | 2.25 | 0.011 | | |
| 4-Methoxybiphenyl | 5.51 | 0.470 | 6.11 | 0.030 | | |
| Diazene oxide | 3.19 | 0.272 | 2.60 | 0.013 | | |
| Carbamate | 9.71 | 0.827 | 7.57 | 0.037 | | |
| Polar | 0.639 | 0.054 | 7.97 | 0.039 | | |
| Unknown (12F; 12S) | 10.6 | 0.899 | 3.71 | 0.018 | | |
| Baseline | 11.7 | 1.00 | 9.55 | 0.047 | | |
| Unextractable | 12.7 | 1.08 | 16.1 | 0.079 | | |
| Soxhlet MeOH | 2.23 | NR | | | 45.3 | 0.005 |
| Soxhlet MeOH/water | 0.70 | NR | | | | |
| Enzyme hydrolysate | 1.22 | 0.104 | 1.67 | 0.008 | | |
| Acid hydrolysate | 0.706 | 0.060 | 1.61 | 0.008 | | |
| Base hydrolysate | | | 10.9 | 0.053 | | |
| Solids | 5.67 | 0.483 | 1.92 | 0.009 | NR | NR |

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
NR = Not reported.
[2] The diazene metabolite, previously identified as D3598, has been determined a residue of concern (in addition to the parent) in plants for tolerance expression and risk assessment purposes (DP# 277089, T. Bloem, 8/16/01).

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**TABLE C.2.2.2. Distribution of the Parent and the Metabolites in Corn Matrices Following Application of [U-anisole ring-$^{14}$C]Bifenazate at 5.0 lb ai/A.[1]**

| Metabolite Fraction | Corn, Forage | | Corn, Stover | | Corn, grain | |
|---|---|---|---|---|---|---|
| | TRR = 65.3 ppm | | TRR = 3.16 ppm | | TRR = 0.0947 ppm | |
| | %TRR | ppm | %TRR | ppm | %TRR | ppm |
| ACN extracts | 76.77 | NR | 54.93 | NR | | |
| ACN/ammonium acetate extract | 1.70 | NR | | | | |
| ACN/MeOH/water extract | 0.90 | NR | | | | |
| MeOH extract | 1.20 | NR | | | | |
| ACN/water extracts | | | 21.42 | NR | | |
| Water extracts | | | 3.31 | NR | | |
| Ethyl acetate extract | | | 0.44 | NR | | |
| -Total extracts | 80.6 | 52.6 | 80.1 | 2.48 | | |
| Bifenazate | 5.42 | 3.54 | 6.11 | 0.189 | | |
| Diazene (D3598) [2] | 11.8 | 7.74 | 10.0 | 0.310 | | |
| Biphenyl-4-yl-sulfate | 3.74 | 2.44 | 8.17 | 0.253 | | |
| 3-Aminobiphenyl-4-ol | 3.84 | 2.51 | 3.27 | 0.101 | | |
| Biphenyl-3,4-diol | 4.29 | 2.80 | 3.04 | 0.094 | | |
| Biphenyl-4-ol | 4.38 | 2.86 | 2.39 | 0.074 | | |
| 4-Methoxybiphenyl-3-amine | 1.28 | 0.837 | 2.56 | 0.079 | | |
| 4-Methoxybiphenyl | 7.92 | 5.17 | 6.43 | 0.199 | | |
| Diazene oxide | 2.70 | 1.76 | 3.05 | 0.095 | | |
| Carbamate | 13.0 | 8.47 | 14.3 | 0.442 | | |
| Polar | 1.09 | 0.711 | 4.01 | 0.124 | | |
| Unknown (12F; 12S) | 7.17 | 4.68 | 9.85 | 0.305 | | |
| Baseline | 9.51 | 6.21 | 10.3 | 0.320 | | |
| Unextractable | 14.2 | 9.27 | 15.8 | 0.489 | | |
| Soxhlet MeOH | | | | | 24.01 | 0.023 |
| Biphenyl -4-yl-sulfate | | | | | 1.44 | 0.001 |
| Polar | | | | | 19.9 | 0.019 |
| Baseline | | | | | 7.80 | 0.007 |
| Soxhlet MeOH/water | | | | | 0.66 | NR |
| Enzyme hydrolysate | 1.64 | 1.07 | 2.14 | 0.066 | 24.7 | 0.023 |
| Acid hydrolysate | 0.65 | 0.424 | 2.78 | 0.086 | 5.06 | 0.005 |
| Base hydrolysate | 6.50 | 4.24 | 8.77 | 0.272 | 31.5 | 0.030 |
| Unknowns | 4.95 [3] | 2.78 | | | | |
| Baseline | 0.239 | 0.156 | | | | |
| Solids | 1.08 | 0.705 | 2.08 | 0.064 | 5.76 | 0.005 |

[1] Shading indicates that the extraction step and/or characterization analysis was not conducted for the matrix in question.
NR = Not reported.
[2] The diazene metabolite, previously identified as D3598, has been determined a residue of concern (in addition to the parent) in plants for tolerance expression and risk assessment purposes (DP# 277089, T. Bloem, 8/16/01).
[3] Eight peaks, each accounting for ≤1.59% TRR (≤1.04 ppm).

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**TABLE C.2.3.1. Summary of Characterization and Identification of Radioactive Residues in Corn Matrices Following Application of [U-anisole ring-$^{14}$C]Bifenazate at 0.75 lb ai/A.**

| Compound | Forage | | Stover | |
|---|---|---|---|---|
| | TRR = 8.52 ppm | | TRR = 0.492 ppm | |
| | % TRR | ppm | % TRR | Ppm |
| Bifenazate | 15.1 | 1.29 | 2.44 | 0.012 |
| Diazene Metabolite (D23-06 aka D3598) | 8.52 | 0.726 | 6.49 | 0.032 |
| Biphenyl-4-yl-sulfate (D23-19 or A1530S) | 4.69 | 0.400 | 11.8 | 0.058 |
| 3-Aminobiphenyl-4-ol (D23-08) | 1.98 | 0.169 | 3.38 | 0.017 |
| Biphenyl-3,4-diol (D-23-13) | 3.43 | 0.292 | 4.22 | 0.021 |
| Biphenyl-4-ol (D23-16 or A1530) | 3.54 | 0.301 | 2.36 | 0.012 |
| 4-Methoxybiphenyl-3-amine (D23-03) | 2.46 | 0.209 | 2.25 | 0.011 |
| 4-Methoxybiphenyl (D23-02 or D1989) | 5.51 | 0.470 | 6.11 | 0.030 |
| Diazene oxide (D23-07) | 3.19 | 0.272 | 2.60 | 0.013 |
| Carbamate Metabolite (D23-04) | 9.71 | 0.827 | 7.57 | 0.037 |
| Polar | 0.639 | 0.054 | 7.97 | 0.039 |
| Unknowns | 10.6 | 0.899 | 3.71 | 0.018 |
| Baseline | 11.7 | 1.00 | 9.55 | 0.047 |
| Soxhlet MeOH + MeOH/water extracts | 2.93 | *0.250* | -- | -- |
| Enzyme hydrolysate | 1.22 | 0.104 | 1.67 | 0.008 |
| Acid hydrolysate | 0.706 | 0.060 | 1.61 | 0.008 |
| Base hydrolysate | -- | -- | 10.9 | 0.053 |
| Total identified | 58.13 | 4.956 | 49.22 | 0.243 |
| Total characterized | 27.80 | 2.367 | 35.41 | 0.173 |
| Total extractable | 89.26 | 7.604 | 93.08 | 0.457 |
| Unextractable (PES)[1] | 5.67 | 0.483 | 1.92 | 0.009 |
| Accountability[2] | 94.9 | | 94.7 | |

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

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**TABLE C.2.3.2. Summary of Characterization and Identification of Radioactive Residues in Corn Matrices Following Application of [U-anisole ring-$^{14}$C]Bifenazate at 5.0 lb ai/A.**

| Compound | Forage | | Stover | | Grain | |
|---|---|---|---|---|---|---|
| | TRR = 65.3 ppm | | TRR = 3.16 ppm | | TRR = 0.0947 ppm | |
| | % TRR | ppm | % TRR | ppm | % TRR | ppm |
| Bifenazate | 5.42 | 3.54 | 6.11 | 0.189 | -- | -- |
| Diazene Metabolite (D23-06 aka D3598) | 11.8 | 7.74 | 10.0 | 0.310 | -- | -- |
| Biphenyl-4-yl-sulfate (D23-19 or A1530S) | 3.74 | 2.44 | 8.17 | 0.253 | 1.44 | 0.001 |
| 3-Aminobiphenyl-4-ol (D23-08) | 3.84 | 2.51 | 3.27 | 0.101 | -- | -- |
| Biphenyl-3,4-diol (D-23-13) | 4.29 | 2.80 | 3.04 | 0.094 | -- | -- |
| Biphenyl-4-ol (D23-16 or A1530)) | 4.38 | 2.86 | 2.39 | 0.074 | -- | -- |
| 4-Methoxybiphenyl-3-amine (D23-03) | 1.28 | 0.837 | 2.56 | 0.079 | -- | -- |
| 4-Methoxybiphenyl (D23-02 or D1989) | 7.92 | 5.17 | 6.43 | 0.199 | -- | -- |
| Diazene oxide (D-23-07) | 2.70 | 1.76 | 3.05 | 0.095 | -- | -- |
| Carbamate Metabolite (D23-04) | 13.0 | 8.47 | 14.3 | 0.442 | -- | -- |
| Polar | 1.09 | 0.711 | 4.01 | 0.124 | 19.9 | 0.019 |
| Unknowns | 12.1 | 7.46 | 9.85 | 0.305 | -- | -- |
| Baseline | 9.75 | 6.37 | 10.3 | 0.320 | 7.80 | 0.007 |
| Soxhlet MeOH + MeOH/water extracts | -- | -- | -- | -- | 0.66 | *<0.001* |
| Enzyme hydrolysate | 1.64 | 1.07 | 2.14 | 0.066 | 24.7 | 0.023 |
| Acid hydrolysate | 0.65 | 0.424 | 2.78 | 0.086 | 5.06 | 0.005 |
| Base hydrolysate | -- | -- | 8.77 | 0.272 | 31.5 | 0.030 |
| Total identified | 58.4 | 38.1 | 59.3 | 1.84 | 1.44 | 0.001 |
| Total characterized | 25.2 | 16.0 | 37.9 | 1.17 | 89.6 | 0.084 |
| Total extractable | 89.4 | 58.3 | 93.8 | 2.90 | 85.9 | 0.081 |
| Unextractable (PES)[1] | 1.08 | 0.705 | 2.08 | 0.064 | 5.76 | 0.005 |
| Accountability[2] | 90.4 | | 93.8 | | 90.8 | |

[1] Residues remaining after exhaustive extractions.
[2] Accountability = (Total extractable + Total unextractable)/(TRR from combustion analysis; see TABLE C.2.1) * 100.

## C.3. Proposed Metabolic Profile

The petitioner proposed that the metabolism of bifenazate in corn proceeds via oxidation of the hydrazine moiety of bifenazate to form the diazene (D23-06 or D3598) which is further oxidized to the diazene oxide (D23-07) and then degraded by oxidative/hydrolysis of the side chain yielding 4-methoxybiphenyl (D23-02), 3-aminobiphenyl-4-ol (D23-08), biphenyl-3,4-diol (D23-13), biphenyl-4-ol (D23-16), 4-methoxybiphenyl-3-amine (D23-03), and biphenyl-4-yl-sulfate (D23-10).

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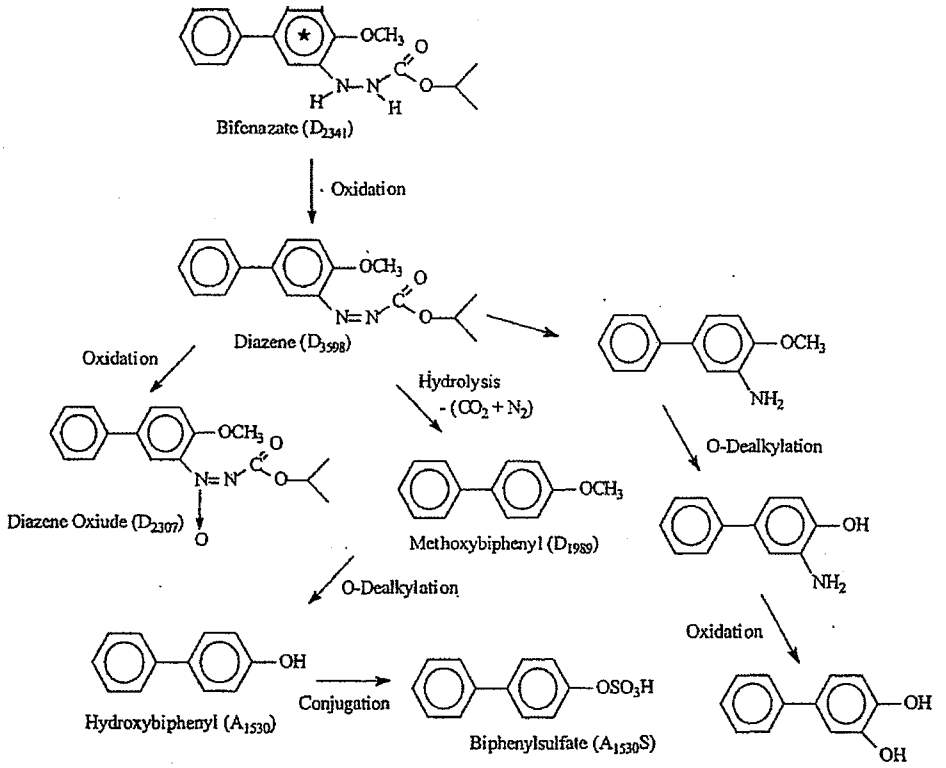
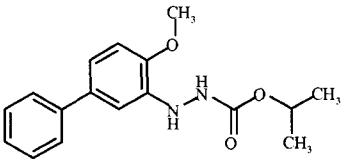
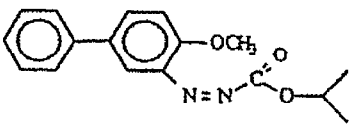
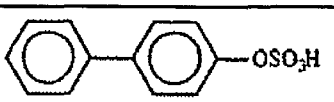
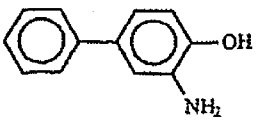
## FIGURE C.3.1. Proposed Metabolic Profile of Bifenazate in Corn




* Denotes the position of radiolabel

**TABLE C.3.1. Identification of Compounds from Metabolism Study**

| Common name/code Figure C.3.1 ID No. | Chemical name | Chemical structure |
|---|---|---|
| Bifenazate D23-01 or D2341 | 1-methylethyl 2-(4-methoxy[1,1'-biphenyl]-3-yl)hydrazinecarboxylate |  |
| Diazene Metabolite D23-06 or D3598 | isopropyl (*E*)-(4-methoxy-1,1'-biphenyl-3-yl)diazenecarboxylate<br>or<br>diazinecarboxylic acid, 2-(4-methoxy-[1,1'-biphenyl]-3-yl), 1-methylethyl ester |  |
| D23-19 or A1530S | A1530-sulfate<br>or<br>Biphenyl-4-yl-sulfate |  |
| D23-08 | [1,1'-biphenyl]-4-ol, 3-amino<br>or<br>3-aminobiphenyl-4-ol |  |

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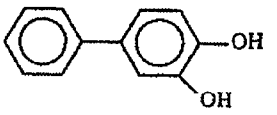
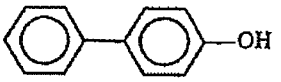
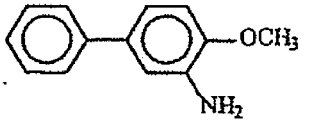
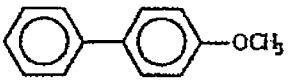
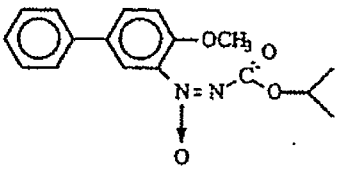
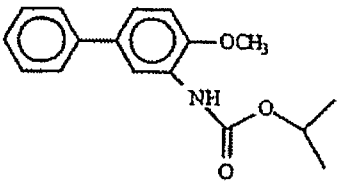
| TABLE C.3.1. Identification of Compounds from Metabolism Study | | |
|---|---|---|
| Common name/code<br>Figure C.3.1 ID No. | Chemical name | Chemical structure |
| D23-13 or D9472 | [1,1'-biphenyl]-3,4-diol<br>or<br>biphenyl-3,4-diol |  |
| D23-16 or A1530 | 1,1'-biphenyl, 4-ol<br>or<br>Biphenyl-4-ol |  |
| D23-03 or D4111 | [1,1'-biphenyl]-3-amine, 4-methoxy |  |
| D23-02 or D1989 | 1,1'-biphenyl, 4-methoxy |  |
| Diazene oxide metabolite<br>D23-07 or D2307 | 3-[(isopropoxycarbonyl)-*NNO*-azoxy]-4-methoxy-1,1'-biphenyl |  |
| Carbamate metabolite<br>D23-04 or D6887 | isopropyl (4-methoxybiphenyl-3-yl)carbamate<br>or<br>carbamic acid, (4-methoxy-1,1'biphenyl]-3-yl)-, 1-methylethyl ester |  |

## D. CONCLUSION

The nature of the residue in field corn is adequately understood. Following over-the-top foliar application of [U-anisole ring-$^{14}$C]bifenazate at 0.75 lb ai/A, TRR in field corn matrices were 8.52 ppm in forage harvested 5 DAT, and 0.492 and 0.0117 ppm in mature stover and grain, respectively, harvested 104 DAT. At the exaggerated application rate of 5.0 lb ai/A, TRR were 65.3 ppm in forage harvested 5 DAT, and 3.16 and 0.0947 ppm in mature stover and grain, respectively, harvested 104 DAT.

Adequate procedures were employed to fractionate and characterize/identify radioactive residues. The following major residue components were identified in/on various matrices following treatment at the nominal rate (the corresponding value for the exaggerated rate treatment is in parenthesis). The parent, bifenazate, was a principal residue in forage at 15.1% TRR (5.4% TRR) and stover at 2.4% TRR (6.1% TRR). The diazene metabolite (designated as D23-06 in the current study but has been determined to be the same regulated metabolite D3598 from previous metabolism studies), was detected in forage at 8.5% TRR (11.8% TRR) and stover

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at 6.5% TRR (10.0% TRR). The carbamate metabolite (D23-04) was present in forage at 9.7% TRR (13.0% TRR) and stover at 7.6% TRR (14.3% TRR). Metabolite D23-19 (or A1530S) was identified in forage at 4.7% TRR (3.7%) and stover at 11.8% TRR (8.2% TRR). Metabolite D23-19 was the only residue identified in exaggerated rate grain at 1.4% TRR.

Other minor metabolites identified in forage and stover included D23-08 (2-4% TRR), D23-13 (3-4% TRR); D23-16 (or A1530; 2-4% TRR); D23-03 (1-4% TRR); D-23-02 (or D1989; 6-8% TRR); and D23-07 (3% TRR). In addition, (4-methoxybiphenyl-3-yl)carbamate was detected in forage (~10-13% TRR) and stover (8-14% TRR) but was determined to be an impurity in the dosing solution.

## E. REFERENCES

DP#: 277089
Subject: ID# 0F06108 - Bifenazate in/on Apple, Apricot, Cotton, Grape, Hops, Nectarine, Peach, Pear, Plum (Prune), and Strawberry. Evaluation of Residue Data and Analytical Methods. Chemical 000586. Case 292702. Submission S575895.
From: T. Bloem
To: T. Levine/S. Oonnithan
Dated: 8/16/2001
MRIDs: 44237801, 45052224, 45052225, 45076505, 45052301-04, 45052311-28

DP#: 313261
Subject: Bifenazate (PC Code 000586). Section 3 Registration of Application of Bifenazate to Apple, Apricot, Cotton, Grape, Hops, Nectarine, Peach, Pear, Plum (Prune), and Strawberry. Petitioner's Response to Deficiencies Identified in HED's Residue Chemistry Reviews (D277089, T. Bloem, 16-Aug-2001 and D288660, T. Bloem, 20-Mar-2003).
From: T. Bloem
To: D. Kenny/S. Oonnithan
Dated: 7/17/06
MRIDs: 46064101, 46064102, 46069801, 46069802, 46069803, 46069804, 46276601, 46180601, 46691301

## F. DOCUMENT TRACKING

RDI: Name1 (Date); Name2 (Date); Name3 (Date); etc.
Petition Number: 8F7373
DP#: 355504
PC Code: 000586

Template Version June 2005

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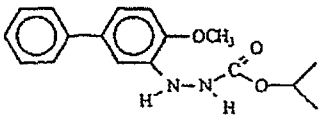
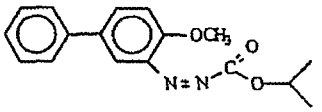
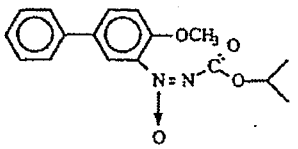
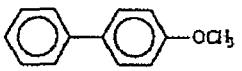
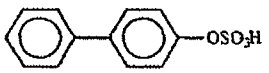
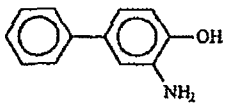
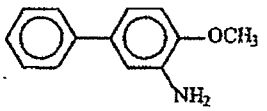
**APPENDIX I. Chemical Names and Structures of Reference Standards Used in the Corn Metabolism Study.** (copied without alteration from MRID 47453707)

| CHEMICAL STRUCTURE | MW | IUPAC NAME | DESIGNATION |
|---|---|---|---|
|  | 300.35 | isopropyl 2-(4-methoxy-1,1′-biphenyl-3-yl)hydrazinecarboxylate | bifenazate<br>D23-01 |
|  | 298.34 | isopropyl (*E*)-(4-methoxy-1,1′-biphenyl-3-yl)diazenecarboxylate | diazene<br>D23-06 |
|  | 314.34 | 3-[(isopropoxycarbonyl)-*NNO*-azoxy]-4-methoxy-1,1′-biphenyl | diazene *N*-oxide<br>D23-07 |
|  | 184.23 | 4-methoxybiphenyl | D23-02 |
|  | 272.25 | Biphenyl-4-yl sulfate<br><br>(obtained as sodium salt) | D23-19 |
|  | 185.22 | 3-aminobiphenyl-4-ol | D23-08 |
|  | 199.25 | 4-methoxybiphenyl-3-amine | D23-03 |

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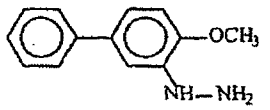
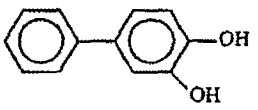
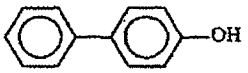
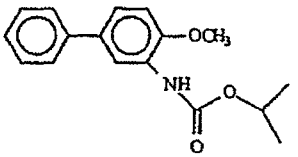
| *(Table I, continued)* CHEMICAL STRUCTURE | MW | IUPAC NAME | DESIGNATION |
|---|---|---|---|
|  | 214<br><br>(250.72 with HCl) | (4-methoxy-[1,1'-biphenyl]-3-yl)hydrazine<br><br>(obtained as the hydrochloride) | biphenyl hydrazine<br>D23-14 |
|  | 186.21 | biphenyl-3,4-diol | D23-13 |
|  | 170.21 | Biphenyl-4-ol | D23-16 |
|  | 285.34 | Isopropyl (4-methoxybiphenyl-3-yl)carbamate | Carbamate<br>D23-04 |